

AMENDMENT**In the Specification:**

Please amend the paragraph appearing at page 1, lines 6-13 as follows:

Relation back and Priority Information

This is a continuation of U.S. Ser. No. 08/466,163, filed on June 6, 1995, now ~~allowed~~ U.S.P. 6,329,509 B1, which is a division of U.S. Ser. No. 08/405,617, filed on March 15, 1995, which is a continuation of U.S. Ser. No. 08/185,899, filed on January 26, 1994, now abandoned, which is a 35 U.S.C. § 371 of PCT/US92/06860, filed on August 14, 1992, which is a continuation-in-part of both U.S. Ser. No. 07/879,495, filed on May 7, 1992, now abandoned and U.S. Ser. No. 07/744,768, filed on August 14, 1991, now abandoned; all of which are incorporated by reference and to which application priority is claimed under 35 U.S.C. § 120.

Please amend the paragraphs appearing at page 6, lines 7-13 as follows:

Brief Description of the Figure

FIG. 1 depicts the sequence of human IgE Fc $\epsilon$ 2 and Fc $\epsilon$ 3 (~~SEQ-ID:SEQ ID NO:~~ 1). This particular sequence is from Padlan *et al.*, Molec. Immun., **23**:1063-1075 (1986). Residues are numbered according to Kabat (*supra*). "X" residues are included to align the Padlan IgE sequence with the Kabat numbering scheme. Sequences which were altered in preparing various IgE mutants are underlined; bold numbers below the lines denote the mutant number.  $\beta$ -strand residues are overlined; loop residues are defined by all residues intervening between two  $\beta$ -strands.

Fig. 2 depicts light and heavy chain sequences for MAE11 (~~SEQ-ID:SEQ ID NOS:~~ 2 and 3), MAE13 (~~SEQ-ID:SEQ ID NOS:~~ 4 and 5) and MAE15 (~~SEQ-ID:SEQ ID NOS:~~ 6 and 7).

Fig. 3 depicts heavy and light chain sequences for HuMae11V1 (~~SEQ-ID:SEQ ID NOS:~~ 8 and 9).

Please amend the paragraph appearing at page 25, lines 4-6 as follows:

Exemplary IgE variants are set forth in Table 5. It will be understood that this table may contain variants that bind to both receptors, differentially to one or the other, or to neither receptor.

Please amend the paragraph appearing at page 37, lines 29-32 as follows:

Each of the IgE specific antibodies was further tested in cell-based and plate assays to select for antibodies which bound to IgE in such a way as to inhibit IgE binding to FCEH and which are not capable of binding to FCEH-bound IgE. The results of these assays are set forth in Table 5b and Table 5a6a below.

Please amend the designation of the Table appearing on page 38 as follows:

**TABLE 56**  
**SUMMARY OF MURINE Anti-Hu mAb CHARACTERISTICS**

Please amend the designation of the Table appearing on page 39 as follows:

**Table 5a6a. Summary of murine Anti-Hu IgE mAb (continued)**

Please amend the paragraph appearing at page 42, lines 9-13 as follows:

g. As a positive control to determine the level of CD 23 on the surface of IM9 cells in each experiment, an aliquot of cells was stained with Becton Dickinson murine monoclonal Leu 20 (anti-CD23) at 10µg/ml for 30 minutes at 4°C followed by 2 washes. The cells were then incubated with FITC conjugated  $\text{f(ab)}_2$  affinity purified goat anti-murine IgG at 50µg/ml.

Please amend the paragraphs appearing at page 43, line 25 to page 44, line 14 as follows:

**EXAMPLE 2**

**Preparation of Variant IgE**

Based on the model of IgE Fc by Padlan & Davies (Mol. Immunol. 23:1063 (1986), which is based on the crystal structure of human IgG1 Fc (Deisenhofer, Biochem. 20:2361-2370 [1981]), a series of mutants were designed which could be used to test the binding of human IgE to its receptors. These mutants are designated Emut 1-13, and are listed in Table 67 below. The Fcε3 domain is comprised of seven β-strands which form a β-sheet structure representative of all immunoglobulin domains; there are six loops which connect these seven β-strands. We refer to these loops by the 2 β-strands they connect, e.g. loop AB connects β-strands A and B. We have constructed mutants of human IgE in which we have substituted five of the Fcε3 domain loops with their counterparts from human IgG1 (Table 67, 1-5). The sixth loop contains the glycosylation site in both IgE and IgG and hence was not altered. One mutant, (Table 67, 6), was made by exchanging human Fcε3 β-strand D with its human IgG1 Fcγ2 counterpart. Seven additional mutants, (Table 67, 7-13), consisted of the substitution of Ala residues into Fcε3 β-strands and a loop in Fcε2.

A human IgE gene was cloned from U266, a publicly available cell line. The gene was cloned into a previously described phagemid vector containing the human cytomegalovirus enhancer and promoter, a 5' intron and sv40 polyadenylation signal (Gorman *et al.*, DNA and Prot. Eng. Techn., 2:3-10 [1990]). Mutagenesis was performed by the Kunkel method (T.A. Kunkel, Proc. Natl. Acad. Sci. USA, 82:488-492 (1985) using buffers and enzymes supplied

with the BioRad Muta-gene phagemid in vitro mutagenesis kit, together with oligonucleotides encoding the human IgG1 sequences shown in Table 67 below. Sequences of the mutant IgE DNAs were checked only at the site of mutation using <sup>35</sup>S dideoxy sequencing.

Please amend the designation of the Table appearing on page 45 as follows:

TABLE 67

Please amend the paragraph appearing at page 48, lines 15-16 as follows:

The foregoing assays were used to determine the ability of the eExample 2 IgE analogues to bind to FCEH and FCEL. The results are set forth in Table 78.

Please amend the designation of the Table appearing on page 49 as follows:

TABLE 78

BINDING OF IGE AND IGE ANALOGUES TO FCEH AND FCEL

Please amend the paragraphs appearing at page 50, lines 1-16 as follows:

Three mutant IgEs exhibited complete loss of binding to the FCEH receptor: mutants 1, 4 and 6. Mutant 6 altered  $\beta$ -strand D at the end of Fc $\epsilon$ 3 close to the Fc $\epsilon$ 2 domain. Mutants 1 and 4 involved alteration of two Fc $\epsilon$ 3 loops which are adjacent and near the Fc $\epsilon$ 4 domain. Note that mutant 7 is subset of mutant 1 in which the three C-terminal residues of loop AB have been changed to alanines (Table 67, 1 vs. 7). However, mutant 7 does not affect binding to FCEH. We interpret this to mean that either 1) Fc $\epsilon$ RI binds at least one of IgE residues 377-381 or 2) the extra residue in IgG1 loop AB (9 residues) substituted for IgE loop AB (8 residues) effected deformation of some adjacent binding determinant, possibly loop EF. That mutants 8 and 10 had no affect on Fc $\epsilon$ RI binding most likely means that the FCEH receptor does not protrude into the cavity bounded by loop AB and  $\beta$ -strand D.

Although mutant 4 had a Leu replacing Gly444 (Table 67), this should not affect the conformation of loop EF. Residue 444 is prior to the N-terminus of this  $\alpha$ -helix. In addition, murine IgE has a Val at position 444 and rat IgE has an Asp. The two buried hydrophobic residues in the middle of the  $\alpha$ -helix, W448 and I449, are retained in the substituted IgG1 loop (W448, L449) as is G451 which terminated the  $\alpha$ -helix. Hence the conformation of loop EF should be similar in IgE and IgG1.

Please amend the paragraph appearing at page 50, lines 30-32 as follows:

In addition to a glycosylation site at Asn430 which corresponds to the glycosylation site in IgG Fc, human IgE contains another glycosylation site at Asn403. Mutant 9 converted Asn403 and Thr405 to alanines (Table 67). Loss of carbohydrate did not affect binding to either receptor.

Please amend the paragraph appearing at page 51, lines 10-14 as follows:

**EXAMPLE 4**  
**Preparation of Humanized MaE11**

Residues were selected from MaE11 and inserted or substituted into a human Fab antibody background (V<sub>H</sub> region Kabat subgroup III and V<sub>L</sub> region kappa subgroup I). A first version, hmae11v1 or version 1, is described in Table 89.

Please amend the designation of the Table appearing on page 52 as follows:

**TABLE 89. Changes in V<sub>H</sub> human subgroup III and V<sub>L</sub> κ subgroup I (Kabat) consensus sequences for humanized MaE11 Version 1**

Please amend the paragraphs appearing at page 53, lines 8-13 as follows:

The affinity of version 1 was assayed and found to be about 100 times lower than that of the donor antibody Mae11 (see Figs. 4a and 4b). Therefore, further modifications in the sequence of version 1 were made as shown in Table 910. Determination was made of the ability of these further modifications to inhibit the binding of labelled huIgE to FCEH.

The 50% inhibition assays whose results are shown in Table 910 were conducted as follows:

Please amend the designation of the Table appearing on pages 54-55 as follows:

**TABLE 910**  
**Humanized MaE11 Variants**

Please amend the paragraphs appearing on page 56, lines 2-12 as follows:

As can be seen from Table 910 and Figs. 4a and 4b, version 8 (in which human residues of version 1 at sites 60 and 61 in the light chain were replaced by their Mae11 counterparts) demonstrated substantially increased affinity. Further increases in affinity are seen in versions 8a and 8b, where one or two murine residues replaced human residues. Other increases, at least virtually to the level of Mae11, were accomplished by replacing hydrophobic human residues

found in the interior of VH and VH1 with their MaE11 counterparts, resulting in the variant designated version 9 (see Table 910 and Figs. 4a and 4b). Accordingly, the humanized antibodies of this invention will possess affinities ranging about from 0.1 to 100 times that of MAE11.

Table 4011 explores the effects on FCEH affinity of various combinations of humanized maE11 IgG1 variants.

Please amend the designation of the Table appearing on the top of page 57 as follows:

**Table 10. Humanized MaE11 IgG1 Variants**

Please amend the paragraph appearing at page 57, lines 8-13 as follows:

**EXAMPLE 5**

**Creation of IgE Mutants**

IgE mutants (Table 4112) were prepared to evaluate their effect on binding to anti-IgE, especially MaE11, and to FcεRI and FcεRII. Some of the mutants were designed to substitute for a specific amino acid residue another residue with either similar or very different charge or size. The impact of these changes on receptor binding is reflected in the table below.

Please amend the designation of the Table appearing on pages 59-61 as follows:

**Table 1112. Amino acid sequences of IgE mutants**